

PHOTOGRAPHY APPLIED TO THE MICROSCOPE.

BY THE SAME AUTHOR.

THE

'ART AND PRACTICE OF INTERIOR PHOTOGRAPHY,'

ILLUSTRATED WITH PHOTOGRAPHS AND DIAGRAMS;

AND
'PHOTOGRAPHY FOR ARCHITECTS,'

A HANDBOOK FOR ARCHITECTS.

LONDON:

ILIFFE AND SON, 3, ST. BRIDE STREET.

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Section of Human Scalp (Horizontal).
x 36 diameters.

6

PHOTOGRAPHY

APPLIED TO THE

20.4

MICROSCOPE,

By F. W. MILLS,

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"Photography for Architects."

WITH A CHAPTER ON MOUNTING OBJECTS, BY

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Author of "An Elementary Manual of Microscopical Manipulation."

ILLUSTRATED WITH HALF-TONE PLATES AND WOODCUTS.

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PREFACE.

The Author, believing that microscopists feel the want of a practical guide to instruct them in the application of photography to their science, has endeavoured to provide requisite instructions by sending out this volume to the world.

He gratefully acknowledges the valuable addition of a chapter on "Mounting Objects," contributed by his friend, Mr. T. Charters White, also the kind assistance which has been afforded him by Mr. V. A. Corbould, in preparing some of the following pages.

F. W. M

Camera Club,

Charing Cross Road,

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Photography applied to the Microscope.

CHAPTER I.

THE PREPARATION OF MICROSCOPICAL OBJECTS.

To obtain a successful photograph of a microscopical subject, not only must the illuminating beam be accurately centralised, but what is perhaps of more importance in the case of such transparent objects as anatomical and pathological sections, they should be mounted perfectly flat all over, presenting one even surface which shall be in strict coincidence with the optical plane of the lens employed. Unless this condition exists it is not possible to avoid the blurred, out-of-focus appearance which an irregular surface presents. The mounters of this class of preparations are scarcely sufficiently aware of the necessity for this flatness, hence their productions, while adequately suitable for ordinary microscopical observation, are often unavailable for photo-micrography by reason of the defect just described. While it is, as a rule, not desirable to interfere with preparations put

* Kindly contributed by Mr. T. CHARTERS WHITE, M.R.C.S., F.R.M.S.

up by a professional mounter, yet in many cases of want of flatness this may be overcome by gently warming the slide and pressing it and the cover glass between the finger and thumb, but such a proceeding must be carried out with extreme delicacy and caution. As the mounting medium is Canada balsam in the majority of preparations, a gentle increase of warmth is sufficient to soften the mountant and allow of the flattening necessary to render the slide suitable for giving a generally sharp negative. The photographer who desires the best results would find the ability to cut his own sections a most valuable addition to his acquirements, as in that case he would produce sections with perfectly parallel sides, not cut too thin, and properly stained, and furthermore, would have an interest in his preparation from knowing its origin and history, being, as it were, in at its birth. With a view to aiding the student to carry these conditions into effect, we shall proceed to give such directions for the cutting of sections of the hard and soft tissues as we may deem useful in guiding him towards the attainment of satisfactory photographic results. Cutting sections of the hard tissues is about one of the simplest operations in microscopical manipulation, and as such need not debar the student from taking it up on the score of his never having attempted such work before.

The hard tissues may be classed under three heads, viz., Animal, Vegetable, and Mineral.

The animal includes such tissues as bone, teeth, and cartilage. The vegetable includes hard woods and the so-called "stones" of fruit. The mineral will be chiefly rock sections and coal. It will be necessary to give details relative to the sectionising of each of these classes. With reference to rock section cutting, it will be sufficient to say that it can only be done by the employment of a lapidary's wheel, and as this appliance is not generally to be found in an amateur's "armamentaria," such sections are more readily purchased, and are, as a rule, fairly thin enough to yield very good negatives. Coal sections are also amongst those that may be more conveniently purchased than made, as they are only made after a great deal of dirty work in grinding them down. Thus we may dismiss from our consideration the subjects of rock and coal sections, and devote a short space to the remaining two heads. Of all the hard tissues, that which, while involving a little more tedious work, produces the most satisfactory result, is bone. While any of the bones of the animal frame when studied in section afford interesting information, yet for demonstration and instruction purposes, we should recommend the *femora* as offering a convenient form for first attempts at sectionising. Thin slices, about one millimetre thick, are cut with a fine saw from this bone and soaked in ether to remove the fat with which the bone is always

permeated. The section to be reduced may then be placed between two plates of ground glass, with water and powdered pumice-stone, and by a rotary movement of the upper plate of ground glass upon the under, the section becomes gradually reduced, and the reduction may be continued till the section disappears in powder; but this contingency must be guarded against by the timely removal of the section to pieces of almost worn-out ground glass, between which, and with *water alone*, the desired reduction can be safely attained, while the section is at the same time polished. The great advantage derivable from this mode of reduction in its relation to photo-micrography, especially if properly carried out, is that such a section must be absolutely flat, and its upper and lower faces ought to be perfectly parallel. When ground sufficiently thin, the section must be cleansed from all foreign particles by a thorough brushing with a camel-hair brush in repeated changes of clean water; it may then be laid on the palm of a clean, dry hand, and its surface moisture removed by the finger. Now, as its substance is saturated with clean water, it may be mounted in moderately stiff Canada balsam without any fear of the balsam running into the structure and obliterating its details.

A pleasing and instructive modification of this plan may be followed by removing the section from the ether after allowing for the thorough

removal of all fatty matter, and transferring it to collodion stained with fuchsine; this follows the ether into all the cavities in the bone after a few days' soaking. It may then be removed and allowed to become quite dry and hard. The section may then be ground down after the plan already described, the water not having any effect on the coloured collodion, and the balsam mountant serving to show up the cavities filled with it. The reduction of teeth to sections is a matter of more difficulty on account of the hardness of the enamel, and unless sections are cut with a lapidary's wheel only one section can be obtained from each tooth. To obtain this the tooth must be rubbed down on one side on a corundum hone with water, that side of the tooth being then polished perfectly and cemented by *hard* Canada balsam to a slip of glass, and then ground down in a similar manner till thin enough, which may be ascertained by repeated trials. The so-called "stones" of fruit may be treated in a similar manner to bone. Sections of the woody tissues may be obtained by cutting thin slices in a microtome if they are in a green and fresh condition, but we should recommend the student to seek among the shavings of the cabinet-maker for those pieces thin enough and smooth enough to show the intimate structure clearly when mounted in Canada balsam, and to be sure to ascertain the names of the various woods from which the shavings are derived. Sec-

tions of the stems of plants may be preserved in methylated spirit till they are mounted. They make very interesting as well as instructive objects if doubly stained, to carry out which the sections may be first steeped in an aqueous solution of a red aniline dye, and when sufficiently coloured removed to an alcoholic solution of an aniline blue, which will push out the red colour in all parts excepting those portions which having a greater affinity for it will retain it. The section may then be cleared by being floated on to a drop of cedar oil, and mounted in balsam. In making sections of the leaves of plants great care must be observed to protect and prevent such delicate structures from being crushed while being held firmly for cutting. Recourse must, therefore, be had to the following plan:—Several leaves may be placed together and held between the two parts of a divided carrot, which being put into the well of a microtome may be sectionised as a whole, the sections of leaves being afterwards floated in water to separate them.

We now come to the consideration of what is the most interesting as well as the most difficult process of section-cutting, *i.e.*, that of making sections of anatomical and pathological tissues, for these, in their natural condition, are too soft to allow of being cut sufficiently thin for microscopical examination, therefore the material must be previously brought into such a condition of firmness as to permit of its being sliced. There are two

methods of accomplishing this—they are by freezing the tissue, and by immersion for a time in hardening fluids. In adopting the first of these processes, the tissue to be cut should be allowed to soak in gum mucilage till this has run into and permeated its structure, and then frozen on to the plate of the microtome, as will be subsequently explained. The author prefers this process because the tissues are more likely to retain their true histological characters than if they had been passed through various hardening solutions. The freezing can be accomplished by placing the tissue to be cut in a paper cup previously filled with the mucilage, and placed in a freezing mixture of ice and salt, or by the more convenient use of a freezing microtome, such as Cathcart's, in which the congelation is produced by an ether spray directed against the under side of the small plate of that instrument; in either case the razor or other cutting instrument must be kept cold in iced water. The foregoing method, when used for small or medium sized objects, cannot be surpassed for speed or success, and is therefore strongly recommended. The second process of hardening material is by the use of such fluids as coagulate the albumen contained in the animal tissues. Of these, the handiest is absolute alcohol, which by abstracting the water from, also coagulates the albumen in all animal tissues submitted to its action; but while it does this with much rapidity, there is also the risk of its

producing shrinkage, and consequent distortion of the histological elements, therefore some other agent must be employed by which these histological elements may be fixed at once while the initial steps of the hardening are being produced. All animal tissue, to be seen in its normal condition, should be immersed as soon after death as possible in some fluid which should fix these cellular elements, such as Flemming's fixing fluid, which is thus made:—

Chromic acid of 1 per cent. strength	25	volumes.
*Osmic acid	10	"
Acetic acid	10	"
Water	55	"

This is probably the most convenient fixing agent the author has employed. The best results are obtained when a small cube of the material, about $\frac{1}{2}$ in. wide, is allowed to soak in this fluid for about an hour, or if not convenient to be cut at that time, it may remain for some days without injury or detriment to the tissue worthy of consideration. Corrosive sublimate is also a valuable coagulant of albumen, thus acting energetically as a fixing agent, but there is afterwards a great deal of trouble involved in the removal of the mercuric salt, which, unless entirely eliminated, leaves stains of a most annoying character, therefore the author would not recommend this fixing agent to the student, but advise him, at any rate at first, to confine himself to the use of "Flemming."

*See "The Microtomist's Vade Mecum," by A. Bolles Lee, page 23.

Supposing, now, the substance to be cut be sufficiently firm to be handled without bending, it must be supported on all sides by being imbedded in some plastic material, such as paraffin wax, or as celloidine. Paraffin wax may be obtained of different melting points, and it is useful to melt these in varying proportions till the mass on cooling presents the same degree of hardness as the cube of animal tissue to be cut. This may then be placed in a small paper cup, and the melted paraffin wax poured over it, and set aside in an equable temperature to harden, when the paper may be peeled off the block, and this stuck with melted paraffin to the object-holder of the microtome ready for cutting. In the case of tissues of a very cellular character, such as the lungs, a process of paraffin infiltration may be resorted to. Thus, all watery fluids having been abstracted by the use of absolute alcohol, chloroform may be substituted, the tissue being allowed to soak in this till thoroughly permeated; some paraffin wax may now be dissolved in chloroform in a test tube by means of a gentle heat, and the tissue placed in this and kept at a gentle temperature till the melted wax fills the structure, when it may be removed and set aside to cool. The subject of section-cutting is such an extensive one that several volumes have been devoted to its treatment, and therefore it is a matter of considerable difficulty to give directions that shall be full and at

the same time concise enough for a manual of this description; but there is one other plan of infiltration and imbedding which yields such good results that it claims inclusion in any list of methods by which suitable objects for photographic purposes can be produced—it is that of infiltration with celloidine. In a previous part of this chapter, the infiltration of bone and the osseous tissues by means of coloured collodion was treated very fully, and the application of that process to hardened tissues being the same, less the fuchsine, it is not necessary to do more than suggest its use in those cases where it is desirable to support, both internally and externally, structures so loose as to render them liable to destruction in the act of cutting them thin enough to furnish suitable sections.

Nothing now remains but to give a few brief directions for mounting such sections. Staining these will be found a valuable adjunct, not only in differentiating the various elements in a section, but in also affording a more instructive slide in photographing the subject, but it must be premised that all colours do not lend themselves equally well to actinic action, therefore, in staining a section, this fact must be borne in mind. According to general experience the pale lavender stain given by very weak logwood solutions is the most difficult of any dye to obtain a good result with while a deep red stain is an almost inaccessible

barrier to the actinic rays, and between these two colours only isochromatic plates can render a faithful contrast; but as ordinary plates will be most generally employed, we might say that sections stained with borax carmine, bismarck brown, and methyl violet, if not too deeply stained, ought to give very favourable results. The staining reagent having been decided on, the section is placed in a rather weak solution of the dye and allowed to remain in a sufficient time to be slightly coloured; for some microscopical purposes the dye may be deeper at first and reduced afterwards, but for photo-micrography much judgment must be exercised in selecting the depth of tint most favourable—that most profitable being one which will give a good contrast against the general background of the field. This depth of stain decided on, the section may be dehydrated by being placed a few minutes in absolute alcohol, and having been drained from that, the section may be laid on the top of a drop of cedar oil, when, the alcohol evaporating, the oil will take its place, and the section will be cleared, after which it may be drained from the oil and mounted in Canada balsam. If now all these stages of hardening, infiltrating, cutting, staining, clearing, and mounting be carefully carried out, a great advance will have been made towards the attainment of a faithful and correct photographic representation of microscopic detail.

CHAPTER II.

MICROSCOPICAL APPARATUS.

It is generally believed that the practice of photo-micrography necessitates the employment of expensive apparatus, constructed specially for that purpose. This is by no means so, as any microscope with any ordinary English objectives may be employed, and the cost of the necessary photographic apparatus and materials ranges from £2 to £3. This statement must, however, be qualified to some extent in many cases; for instance, if it is desired to photograph some extremely minute objects, such as certain diatoms, bacteria, etc., a high power objective in conjunction with a substage condenser will be required.

Microscopes that are most suitable for our purpose are those in which the body-tube may be bent back until it becomes parallel with the table on which they stand, which have their body-tube of large diameter, and, for high power work, a good, fine adjustment is absolutely necessary for obtaining a well focussed image. The author considers the form adopted in Watson and Sons' microscopes the best. He has used one of their "D" Edinburgh Student's Microscopes for a considerable time, and fails to perceive the

slightest deterioration as the result of wear, even with high power objectives. A thin stage is often of advantage, as it allows very oblique illumination to be employed. A substage diaphragm, and an Iris diaphragm placed above the objective will be found most useful under the most common

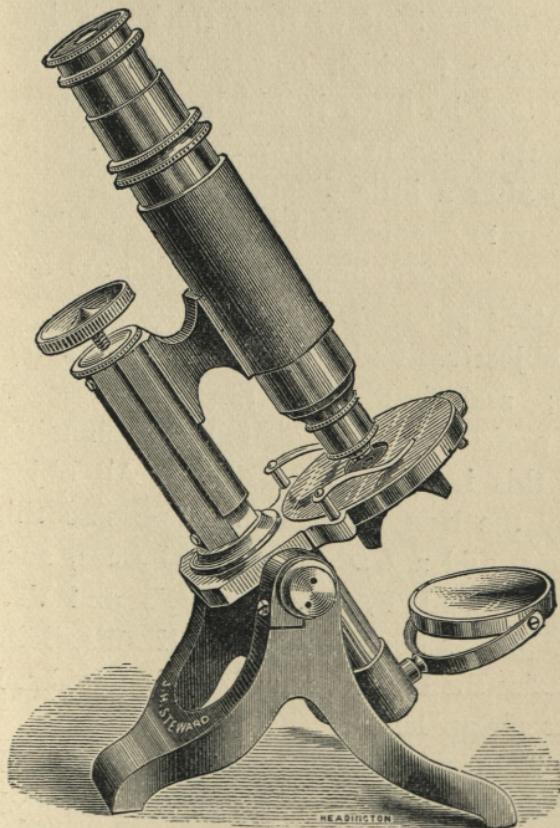


FIG. 2.

circumstances. From what has been said, and from the description of accessory apparatus to follow, it must not be imagined such are neces-

sary, except in unusual cases or special branches of photo-micrography. (The microscope shown in fig. 2 will answer most purposes, with a bull's-eye and paraffin lamp.)

Mechanical stages are not great advantages to photo-micrographers, who work only with low powers, on account of their thickness, but are indispensable for high power work. If a thin sliding stage be fitted it will be found simpler to manipulate (even in conjunction with the highest powers) after a little practice.

A separate chapter will be given explaining the use of other optical apparatus which are used for illuminating purposes.

The objectives and eyepieces must now be considered; and first as to the objectives which should preferably be corrected for photography, though this is not a necessity.* All objectives made by the best English firms possess this requirement, and a few Continental ones, such as Zeiss's DD and F, Prazmowsky's and Verick's numbers 3, 6 and 8, and some of Kraft's, Seibert's and Leitz's; the latter's $\frac{1}{2}$ -inch oil immersion is an excellent cheap lens.

The dividing French objectives, known as "French-buttons," are worthless for photo-micrography, as their *visual* focus is not coincident with their *chemical* or *actinic*, besides

* Dr. Woodward states "the objective selected should always be specially corrected for photography."

which the author never saw any which were accurately corrected for either chromatic or spherical aberrations. Any good optician will correct an objective for photography at a cost of about ten shillings, by adding a supplementary "back" lens of double concave curvatures.

The magnifying power of objectives is spoken of as "*amplification*." This is calculated upon the standard of a single magnifying lens of a given focus—*e.g.*, an objective designated a "one inch" implies that it has the same amplifying power of a single lens of that focus; it does not imply that the objective focusses one inch from the object on the stage of the microscope.

Flatness of field is a point worthy of notice, and more particularly so to the photo-micrographer, as it is most important that his objectives should define the edges at the same focus as the centre of an object.

Penetration (known by photographers as "depth of focus") has been in the past a much-discussed question. Professor Abbé was the first to solve this mystery. Objectives with comparatively small angular apertures have greater penetration than those of wide angle, and as angular aperture increases penetration decreases. Penetration may be designated as a power of clearly showing objects varying in thickness at a given focus. It will be found in practice that an objective of wide angular aperture, if employed in conjunction

with a comparatively small substage diaphragm, will give remarkably more depth of focus than one of low angle without a diaphragm, and will therefore be of far greater value for practical work.

The human eye has a certain power of accommodation, rendering the view represented by the microscope more generally clearly defined than the photographic plate.

The unit employed for measuring microscopic objects is the *micra* (written μ). This almost inconceivably small standard is equal to $\frac{1}{25400}$ part of an inch (being .001 of a millimetre).

The following table will prove of some assistance in selecting the most suitable objective to clearly represent uneven objects of a known thickness in micras:—

Objective.	Air angle.	Aperture.	Penetration power $\frac{1}{a}$.	Focal depth in μ .
4 in.	8	.07	14.30	522
1 $\frac{1}{2}$..	20	.17	5.75	69
$\frac{1}{2}$..	70	.57	1.74	6.3
$\frac{1}{6}$..	100	.76	1.31	1.57
$\frac{1}{12}$..	144	.97	1.02	.61

From the above table it will be seen that the smaller the angular aperture the greater will be the penetration possessed by the objective. For further information, the reader is referred to Mr. G. E. Davis's excellent work, "Practical Micro-

copy,"* by the aid of which the author has been enabled to give the above table. Professor Abbé's paper, published in the *Journal of the Royal Microscopical Society* for August, 1881, will reward those who peruse it, as it contains much valuable information upon this subject.

It is evident that no objective can *clearly* define any object occupying two or more planes, therefore, if a general comparative clearness be obtained of several planes at one focus, it is because the objective be focussed midway between the extreme planes.

It is better to define sharply but one plane than several indistinctly, and accommodation should be strenuously avoided.

The resolving power of an objective is of primary importance; it may be briefly described as the power which a lens possesses of giving clear definition, separating minute particles with accuracy. This power depends absolutely (presuming the objective to possess accuracy of the corrections for sphericity and chromatism) upon the aperture, resolving power increasing in direct proportion with such aperture (numerical aperture is here meant, not angular). "The old method of designating the angular aperture of an objective by the number of degrees in the angle formed by the most lateral rays meeting at the focal point of the objective, has been superseded by the more correct and con-

venient method of calling the capacity which an objective possesses for collecting the whole pencil of rays from an object in air, unity, then commencing with the lowest numbers; this reaches as far as 1.52 for oil immersions. This is called the numerical aperture, and N.A. is written after the numbers to distinguish it. It may easily be found by multiplying the sine of the semi-angular aperture by the refractive index of the fluid used."*

Angular aperture is greatly increased by causing the illuminating rays of light to traverse the object at a very oblique angle. Professor Abbé was the introducer of the system of computing the aperture by the N.A., as above explained. According to his theory (which is quite in accordance with the optical laws at present acknowledged) the corresponding

Air angle is	180 degrees
Water ,,	97 "
Balsam ,,	82 "

To avoid confusion he surmounted the difficult question by employing his term of N.A., being equivalent in all three instances to 1.0.

The ocular, or eyepiece, must now be considered. It consists primarily of two plano-convex glasses, having a diaphragm at about midway between them, each of the curved sides being turned towards the objective, the lens near the eye being named the eyepiece, and the one near the

* *Photo-micrography*, by A. C. Malley. London: H. K. Lewis.

objective the field-glass. The latter increases the usual field, the former magnifies it. The field-glass is employed to alter the course of the rays from the objective, and to form the image at the diaphragm, which cuts off the outer rays arising from spherical and chromatic aberrations ; the image thus formed is again magnified by the eye-piece.

Oculars constructed for photo-micrography differ in their construction from the above, in having instead of plano-convex lenses, plano-concave, cemented to double convex lenses.

Oculars are spoken of as being either shallow or deep, the former having a flatter field-glass than the latter. Shallow ones are preferable for photography, as they give better definition, though they possess less magnifying power. Their amplifying power is designated in England by the letters A, B, C, etc. ; in America in a more comprehensive manner, viz. :—

2 inch	=	A
1½ "	=	B
1 "	=	
¾ "	=	D

All the English opticians do not designate their oculars by the same letter which are of equal amplifying power. They seem, however, to be practically agreed as to the A, and approximately so as to the B and C, but the D, E and F vary very considerably.

If the ocular be employed, it should be corrected for photography, or the visual and actinic foci will not be coincident. For very high power work ($\frac{1}{25}$ in. and $\frac{1}{50}$ in.), it is better to work without it; for medium and low powers, it is an advantage, as it gives better definition, and the amount of light which it cuts off is by no means an inconvenience, as would be the case with high-power work.

Ordinary objectives which have been made some years will generally be found to be over-corrected, and as oculars not corrected for photography will be found to be under-corrected, the lens *may* produce the desired sharp image. An eyepiece is by no means absolutely necessary, as will hereafter be explained.

M. Viallanes has recommended* the employment of a bi-concave lens of shallow curvature, as by this means a very large image will be obtained without a long extension of the camera.

The use of an ocular in photo-micrography has the great advantages of reducing reflections, enabling a short focus camera to be employed.

Before closing this chapter one point which in practice will be found worthy of attention, is that the body-tube of the microscope employed should be lined with black velvet, to absorb any reflected light, or flare spots are certain to present themselves in the photographs.

**La Photographie appliquée aux études d'anatomie microscopique*, page 18.
Paris : Gautier-Villars.

CHAPTER III.

ON THE CHOICE OF PHOTO-MICROGRAPHIC APPARATUS.

This is not an easy matter to advise a beginner upon, as it is not certain in what way he may intend to take up the subject, also to what extent he is prepared to meet it financially. Should the student be unprovided with a microscope, and not wish to attempt work with high power objectives, there is a small piece of apparatus which can be screwed into the flange of his camera, which is to all intents an adapter, into which his low power objective can be screwed,

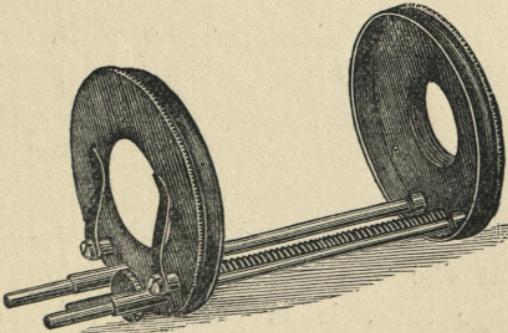


FIG. 3.

having two parallel bars projecting at right angles to the camera front, which support a small light stage that holds the specimen in front of the objective. This apparatus will easily be understood by glancing at the accompanying figure (3).

But it will be gathered that this apparatus is but a makeshift, and cannot possibly be used with objectives of higher power than an inch.

Another apparatus, consisting of a small light camera affixed to the eyepiece of a compound microscope, is upon the market, and is figured in (4). The attachment it is obvious is not

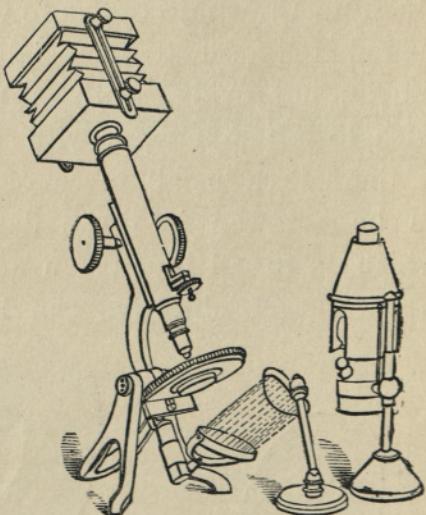


FIG. 4.

very secure, and the weight of this instrument acting at the end of a lever as long as the microscope tube, it is perfectly evident is not calculated to improve the fine adjustment of the microscope; and then, secondly, it is also plain that the instrument cannot possibly be considered as free of vibration during the operation of drawing the shutter of the dark slide, or for the matter of that during exposure altogether. The image also

is but small, owing to the small extension which it is possible to obtain. Having mentioned these two as samples of cheap apparatus, we will now proceed to describe an apparatus which can be used alike for high and low power objectives. It consists of a camera, microscope and fittings and illuminant laid out upon a baseboard so that the whole system having for once and all been "centred" that trouble is to a great extent obviated during subsequent proceedings. Perhaps it will be best to describe the board first and then the apparatus which it carries.

The *Board* is made of well-seasoned wood, and is about 5ft. 6in. or 6ft. in length, having at one end a turntable, which is pivoted about its middle, carrying the microscope, condensers, screens and illuminant, the advantage of this turntable being that the whole of this section of the apparatus can be swung out at an angle from the axis of the camera, so as to enable the operator easily to "find" the portion of specimen he desires to depict, and having found it, to again place the apparatus of this section, as a whole, back to its proper centring, this being secured by a stop being adjusted to this position. At the opposite end of the board is a raised stage about 40 inches in length, upon which the camera slides to and fro for focussing, the stage being of such height as to bring the centre of the plate when in position in the camera in the same optical axis as the

microscope and illuminant. On the right hand side of the board passes a long metal shaft which is cut and keyed at a point over the edge of the turntable. This shaft is supported on wooden columns, and is for moving the fine adjustment of the microscope, which it does by means of a continuous band which passes from it to the microscope.

The *Camera* may be of wood, cardboard, or of the ordinary bellows form, as represented in fig. 5. The bellows should allow of long extension, about 40 inches. No swing-back is required to the camera, and no rising front. There should be two focussing screens, one of ground glass as usual, the other of plain plate glass; the former being suited for roughly getting an idea of the general contour of the specimen, the latter is used merely as a support for a focussing eye-piece used for focussing the aerial image. This eye-piece is adjusted so as to be in focus for a small object placed upon the opposite side of the plate glass when the eye-piece is held in contact with the other surface of the glass. Just in front of the focussing screen a groove should be cut, permitting of tin plates to be used as masks to give a clean edge to the photograph. The most suitable size camera for the above apparatus would be half-plate, $6\frac{1}{2} \times 4\frac{3}{4}$ inches.

The *Microscope*.—If this is being obtained especially with a view to use in photography, it

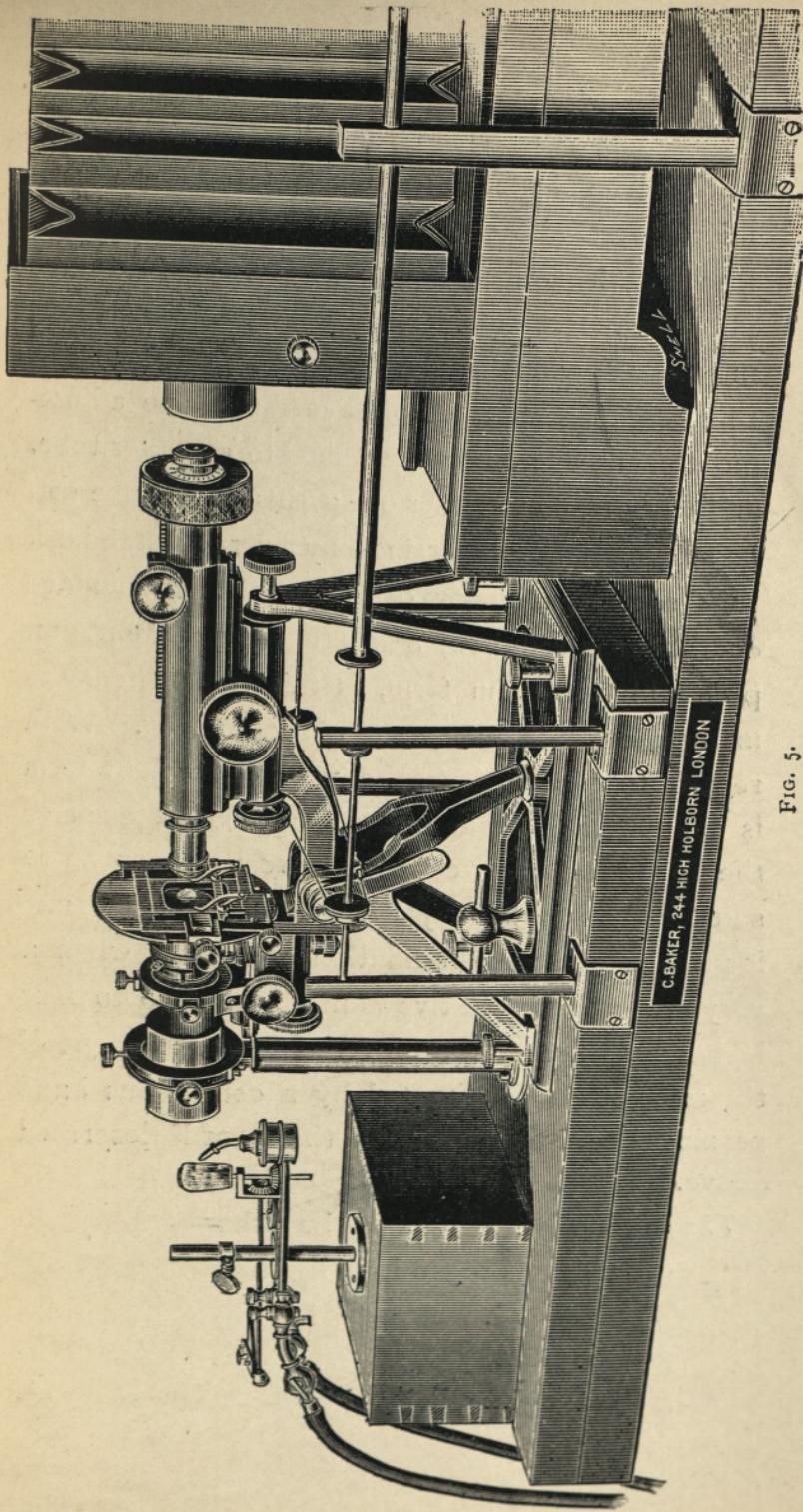


FIG. 5.

ought to be of special construction, the body of the microscope being supported from the middle on a firm tripod. But in all probability, the student of photo-micrography is already in possession of a microscope which he desires to have adapted. In this case it is as well to have the coarse adjustment of the microscope attached to an outer tube, and working on an inner one; this outer tube can be supported at its extremity by a vertical standard, thus securing rigidity of the whole. A substage condenser is required, and a substage diaphragm, preferably an Iris in form, as this is more easy to manipulate without chance of uncentring. With regard to the condenser, a very convenient form is composed of three lenses, superposed, each of the upper two being capable of removal, so as to suit for lower power objectives. A ground glass cap is provided for the illumination of objects when a very low power objective is used, say two to four inches. The wheel working the fine adjustment of the microscope is actuated by a continuous band passing over the shaft on the board described above.

The illuminant. This may be either—

- (α) Oxy-hydrogen lime light.
- (β) Coal gas.
- (γ) Paraffin.
- (δ) Daylight.

The oxy-hydrogen light is probably the most convenient for the majority of workers, two forms of jet being available—

(a) The “blow-through.”

(b) The mixing jet.

The former is the safest for beginners to work with, the oxygen gas being blown through a hydrogen flame upon the lime cylinder, but the light is not so good as that obtained from the latter, in which the oxygen and hydrogen are mixed in a chamber previous to consumption at the nozzle. The nozzle of this jet should be fine in bore, rather finer than when for use in the lantern ; by this means a nearer approach is obtained to the theoretical “point of light.” A very good mixing jet, invented by Mr. Andrew Pringle (made by Messrs. Newton & Co., of Fleet Street, London), is provided with a special cut-off tap, which in one movement cuts the oxygen off completely, and turns at the same time the hydrogen down to a minimum. The limes should be of the hardest description. “Nottingham” limes being the best we have tried. All good jets are provided with an arrangement for turning the limes when they become pitted, for if their use be continued in this state, the flame is liable to spurt sideways, and so crack the condenser. The gas for the jet can be obtained compressed in steel cylinders. In our own case we use the oxygen from a cylinder provided with a Beard’s regulator

which renders the adjustment of the flame possible at the taps of the burner; but take the hydrogen from the mains—or rather, we ought to say, we use coal gas for hydrogen—the only danger in this method of working is that the oxygen must be turned on carefully, as otherwise it may be blown round into the mains and thus extinguish the light at the jet, and form into the bargain a pleasant surprise to the next person who attempts to light the gas in another part of the house; but with care this cannot happen.

Using ordinary coal gas alone, as an illuminant, we have used an Argand burner with fair success, but found it not so good as a Welsbach flame, which is a Bunsen flame playing upon the interior of a cap made of cotton fibre netted and coated with a mixture of metallic salts, chiefly oxide of zirconium. We have also at a press used a common fish-tail burner screwed into the position of the nozzle of the mixing jet, this device being used when we ran short of oxygen, but it is difficult by this means to obtain a good enough light to work with any but very low powers.

(γ) An ordinary paraffin microscope lamp is also available, but is more suited for low powers than high.

(δ) Daylight requires special arrangements scarcely within the reach of ordinary amateurs, a heliostat is needed to keep the sunlight upon the condensers. The heat from this illuminant must

be cut off by using ammonio-sulphate of copper cell.

In our own practice we use a four-inch condenser out of an optical lantern to collect the rays from the source of illumination, and so arrange it that it throws a converging pencil of rays, the apex of this cone being in the diaphragm of the microscope. When coloured screens are used they are placed between the light and this four-inch condenser, this position having two advantages which are:—

- (a) Small imperfections in the glass are of no consequence.
- (b) If the flame should spurt sideways it is better to lose this glass than an expensive condenser.

Before closing this chapter it should be mentioned that in order to do away with vibration of passing traffic, &c., &c., being transmitted to the instrument, we place it on two rolls of cotton wool, one supporting each end of the microscope board.

CHAPTER IV.

ON THE DARK ROOM AND ITS FITTINGS.

Although development may be carried out in the bedroom at night, or in a cupboard, under stairs, or wine-cellar, and such like places, these are obviously inconvenient and quite unsuitable to any systematic scientific work being done. A room should be fitted up specially for the purpose, and possibly the most convenient will be to have the means of converting the room in which the apparatus stands into the dark room—in other words make the dark room of such size that the apparatus can be kept *in situ* in it.

The room should be well ventilated, this being a very important item, as when artificial light is used the air gets very foul, especially when ammonia is being used. It should also have a good water supply, and a sink, over which development can be carried out.

The Illumination.—Gas is the most convenient when it can be obtained. One jet being provided with a by-pass, the pilot flame of which burns behind a collar, thus showing no light when turned down; this does to illuminate the room on ordinary occasions, and for exposing bromide prints, &c.

Another jet is placed inside a lantern glazed with ruby glass; the screen holding glass being removable and replaceable with one of yellow, for use during printing, or the development of plates other than orthochromatic. If gas is not available, paraffin lamp or candles must be made to suffice, but these are not so generally useful as the gas, the heat from it being easily used as a means of ventilating the room. The flue from the lantern should be led out of the room.

A set of about half-a-dozen dishes of porcelain will be found sufficient. Two or three shelves ought to be fitted up to hold the bottles of reagents. The reagents required are as follows, and the following strengths will be found convenient for photographic use:—

Pyrogallic acid*	10 %
Bromide potassium	10 %
Ammonia	10 %
Potass. ferrocyanide	10 %
Quinol	crystalline
Potass. hydrate	in sticks
Potass. metabisulphite	
Sulphite of soda	crystalline
Citric acid	crystalline
Ferrous sulphate	sat. sol.
Neut. oxalate potass.	sat. sol.
Mercury perchloride	sat. sol.
Thiosulphate soda (hypo)	sat. sol.
Powdered alum	sat. sol.

The first three substances on the list are most conveniently kept in dropping bottles of about two

to four ounce capacity. The alum and hypo being kept in stone jars.

Two measuring glasses should be obtained, one of ten-ounce capacity, and another of one drachm. A pair of small balances with glass pans are also required.

The fixing bath should have a wooden lid to prevent dust falling in, it also prevents evaporation when not in use.

A wooden grid should be fixed above the sink to develop upon, this arrangement letting the spillings, &c., fall into the sink below.

CHAPTER V.

EXPOSURE.

We must presume that our readers understand the usual movements of a microscope stand, and pass on to the practical instructions particularly bearing on photography. Having selected an object which presents good contrasts in colour, and does not require a high power objective, say, for instance, the spiracle of a water beetle, place it on the stage. Now screw on to the body a one inch power, place the microscope in a horizontal position, as shown in fig. 6, ready to attach to the camera. The light, whether oil lamp, electric, or any other, must be placed in the exact optical axis of the microscope, together with the condenser. Now focus both the objective and the condenser upon the object placed on the stage. Too much stress cannot be laid upon the importance of having the condenser perfectly centred, whether it be the large four-inch condenser, a wide-angle substage condenser, or an ordinary large bull's-eye. The image of the flame must be exactly focussed in the centre of the field. If it is too much on one side the opposite edge of the field will be dark.

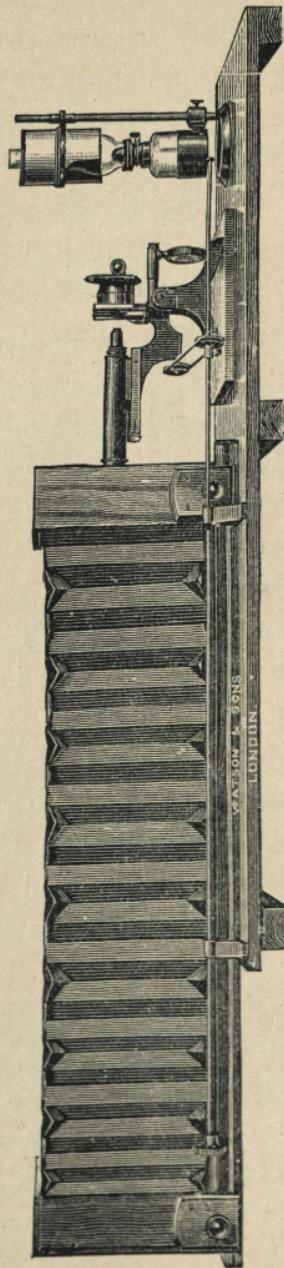


FIG. 6.

Presuming that all is now ready attach the camera, making the joint light-tight with a short tube of black cloth ; extend the camera about 26 to 30 inches ; gently turn the focussing screw while watching the focussing screen made of ground-glass. Having adjusted the focus as sharply as possible with this, remove it and employ the plate-glass in its place. Place a black card in front of the lamp, and insert the double back containing a plate in the camera. Withdraw the shutter, uncovering the plate, and after taking care that the camera no longer vibrates remove the black card from the lamp for, say, one minute, with an ordinary oil lamp having a $\frac{5}{8}$ in. wick ; about twenty seconds will suffice with lime-light. The shutter having been replaced and the slide removed, the plate should be developed as described in the chapter following. Definition may be considerably improved by employing an Iris diaphragm behind the objective, and also behind the stage. This will, however, very materially lengthen the exposure, and several experiments in this direction will well repay the trouble of making them.

If coloured screens are used in conjunction with isochromatic plates, for the purpose of differentiating the colours, the exposure must be proportionately lengthened from about two to seven times the original exposure. The screens should be made either of pot metal or plate glass, coated with a mixture of some dye substance, such as turmeric

or tropœolin, dissolved in collodion until the desired colour is obtained. It is essential, if the glass screen is placed under the objective, that it should be optically true with parallel sides.

The coloured screens are used to differentiate slight differences of colour, such as varying shades of blue, or in another case may be used to render the blues less actinic when they come into relation with reds and yellows, in this case reducing the contrasts.



FIG. 7.
Hipuric Acid. $\times 36$ diameters.

A polariscope may be used to show the structure of certain objects if desired, fig. 7 being an

example, which is given to demonstrate the truth of this statement, as the author has more than once been contradicted upon this point.

It is impossible to give a table of exposures, as different objects require different periods of time depending upon their opacity and the colour of the stain; again, the greater the amplification, resulting from high powers and long extension of the camera, the longer the exposure will require to be. Fig. 8 represents a diatom photographed with half-inch power and A eyepiece, a small stop being used behind the substage condenser. The exposure required was seven minutes, and although much of the sharpness has been lost in producing the illustration, it affords an example of work done with an eyepiece.

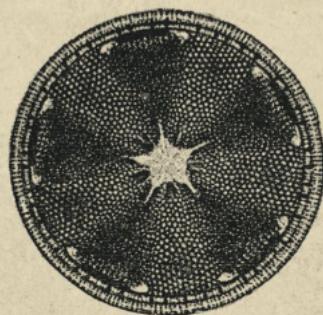


FIG. 8.
Heliopelta Actinoptychus. $\times 140$ diameters.

Opaque objects require very long exposures, and the best results are obtained with objectives of about three to one and a-half inch power. The object may be illuminated with a bull's-eye and side reflector. An exposure meter, such as

Watkin's, has in some workers' hands proved of use.

It is quite impossible to give critical instructions in exposure, nothing but experience being able to instruct the tyro. Having now given him the outline, he must be left to succeed by his own efforts, which, if accompanied with a little care and patience, will reward him by success.

CHAPTER VI.

DEVELOPMENT.

In the last chapter we have seen how the plate is to be exposed. Our attention must now be turned to the process of development, by which the latent image in the film is to be brought to light, thereby making a negative of the plate from which any number of positive copies may be printed.

First, the developing and fixing solutions must be prepared. There are several reagents which act as developers of the latent image. That which is still most generally used is pyro and ammonia, or alkaline pyro. This should be prepared in three stock solutions, viz :—

No. 1.

Pyrogallic acid	1 ounce.
Sulphite of soda	3 ounces.
Water to make	10 ounces.

The sulphite (take care it is pure) must *first* be dissolved in hot water, and when cold the pyro added to the solution, or the pyro ready made up in a 10 per cent. solution may be obtained at a photographic dealer's, under the name of sulpho-pyrogalol.

No. 2.

Bromide of potassium	1 ounce.
Water to make	10 ounces.

No. 3.

Liquid ammonia	880	1 ounce.
Water to make	9 ounces.

These will each be roughly 10 per cent. solutions, consequently, any proportion of the three may be measured out, 10 drops of any one of the solutions containing one grain (or drop of ammonia) of the substance dissolved.

What may be called a normal developer, that is for correct exposures, may be formed by taking of

No. 1	20	minims	} to each ounce of water.
No. 2	10	"	
No. 3	20	"	

Another developer in common use is hydroquinone or quinol, of which the following is a formula :—

A.	Hydroquinone	3 drachms.
	Potassium bromide	40 grains.
	Sulphite of soda	3 ounces.
	Water to make	20 ounces.
B.	Caustic potash	2 drachms.
	Water to make	20 ounces.

Equal parts of A and B are used to develop. This developer has the advantage of not staining the fingers (as does pyro), and may be used to develop several plates in succession.

A third developer which has much to recommend it is eikonogen. This can be bought in a very handy form of cartridges. The contents of a cartridge are dissolved in about six to eight ounces of water, and the developer is at once ready. This has the advantage of saving trouble. It

leaves no scope for varying the constituents of the developer to suit special cases, but this is, perhaps, of less importance in photo-micrographic work where the exposure may be repeated to suit the developer used. The same eikonogen developer may be used for many plates in succession, but gradually loses its power.

One of the above-mentioned developers having been chosen (we recommend the latter for beginners, but personally prefer pyro), the fixing bath must also be mixed and placed ready for use in a tray *kept exclusively for that purpose*.

The strength of the bath should be about—

Hyposulphite of soda	1 ounce.
Water	6 ounces.

A rough idea of the object of the developing and fixing solutions may be given as follows:— Wherever light has acted on the plate through the objective forming the image, there the developer causes a visible reduction of silver as a black deposit, and the intensity of this deposit varies according to the intensity of the light which that part of the plate has received. Dark portions in the object will, therefore, remain white or creamy, and the highest lights of the object will, in the negative, be black. The intermediate tones will be represented accordingly, and so a representation reversed in its lights and darks, or, as it is called, a negative is produced.

The fixing solution: Hypo has the peculiar property of dissolving out all the salt of silver (that part which was not acted upon by light) in the film, and has no power of affecting the reduced image or black portions. Consequently, the shadow portions are, after fixing, more or less clear glass.

Let the beginner remember that the aim in photo-micrography is to expose and develop in such a manner as to obtain a negative which shall give in the subsequent print all the detail of the original and shall show, as far as possible, the same gradations of light and intensity of colour. To approach correct relations of colour the photo-microscopist must have used, in exposure, orthochromatic plates and coloured screens (as we have already pointed out) and in such a case only rather *deep red light* may be used when the plates are taken out of the slides and developed. If ordinary plates have been used, orange light is to be preferred as being quite safe, and as giving a much brighter light by which to see the development. Ruby paper or orange paper form excellent media. Now, as to the actual operations and judgment needed in developing. Having the fixing solution to hand in one tray, remove the exposed plate from the dark slide, and place it film (the duller side) uppermost in the other tray. Now pour the developer on it in such a way as to cover the whole plate quickly and evenly. Rock

the plate gently so as to keep the developer moving ; otherwise, fatal markings with some developers may be caused in the negative. If the subject have been correctly exposed, the image will shortly appear, the high lights of the image coming first, and the whole gradually growing in detail and density. Now comes the necessity for experience and judgment, though there is no reason why the very first plate developed by a beginner may not be one of his best. The difficulty is to decide whether the plate has been over or under exposed, and then, more important still, to know at what stage to stop the development. Now, if the normal developer be used and the temperature always kept the same, the time that the image takes in appearing is some criterion of the exposure. If over-exposed the image comes quickly, if under-exposed it is longer in appearing. But the better way of judging is to watch the character, rather than the time of appearance, of the image. If black patches appear in certain parts, the rest remaining white, then *under-exposure* is to be feared, and a plate much under-exposed is useless to the microscopist. If the plate shows a tendency to *grey all over* then the subject has had *too much exposure*, and immediate steps should be taken to make the best of such a state of things. These we shall indicate immediately. Judging by surface appearances, however, is somewhat misleading with some makes of plates, and, as far as ultimate

density is concerned, practically useless. The only safe guide is to examine the plate by transmitted light, holding it up to the red or orange light. The same rule holds good here. If the plate comes black in patches it has been under-exposed, if thin and grey all over it has been over-exposed; some allowances, of course, being made for what may have been the actual character of the contrasts in the object photographed. The plate which comes rather grey all over and thin at first need never be despaired of. It must be remembered it is the print that has to be considered, not the mere appearance of the negative. There are golden rules here to be borne in mind. If the plate has evidently been under-exposed, and comes harsh in contrast it should not be developed far, or else none of the subsequent printing processes will give even a presentable print. If the plate show the evidences of over-exposure, the development must be prolonged until the image is very dense—until, in fact, very little sign of image can be seen in the blackness. In such a case, if good thick emulsion plates have been used an excellent, though slow, printing negative may still be obtained. This is the great difficulty in developing—to know when to end it. A few experiments with the particular dark room light, plates, and printing method which the beginner may have chosen to use, will soon give him some idea.

It is usual to vary the constituents with the pyro-ammonia developer, if the plate comes up as if under-exposed, the normal developer is poured off and washed out, and one containing, say, 1 grain of pyro, $\frac{1}{4}$ or $\frac{1}{2}$ grain of bromide, and 20 drops of 10% ammonia *per ounce* of developer is applied. Even more ammonia may be added if the plates seem able to stand it. If the plate shows over-exposure, the normal developer is discarded as before, and one containing, say, 4 grains pyro, 2 of bromide, and 1 drop of ammonia per ounce is employed, and the result patiently waited for, as it will come very slowly. Above all, let it be remembered to develop over-exposed plates to very full density, for good printing purposes.

When development is considered complete the plate is well rinsed, and, if thought desirable, cleared of pyro stain by immersion in an alum bath (saturated solution) for two or three minutes. Alum, however, is not altogether a desirable friend, and if the plates have no tendency to frill a weak citric acid clearer (1 in 80) is better. After the alum or acid the plate is again well rinsed, and is then placed in the fixing bath for about ten or fifteen minutes. The plate may be examined from the back, and if every vestige of the white appearance is gone a few more minutes will complete the fixation. The hypo bath may be used for many plates, but it is not advisable to allow it to get very discoloured or overcharged.

with silver. The plate is now to be very thoroughly washed. This, after a preliminary rinse, may be done in daylight, and if in running water, one hour or even less, is sufficient; or six or seven changes of water, made at intervals of about ten minutes, will be found to suffice. The plate, after washing, should be cleaned at the back (the glass side) and placed in a rack, or leaning against a support to dry naturally. When dry it is ready for printing, or it may be varnished as a protection against the possible danger of damp silver paper, which causes red stains in the film almost impossible to remove.

Should the plate prove too thin, it may be intensified. This may be done by placing it in a 1 in 20 solution of mercuric chloride. This will bleach the film, and the bleaching should be allowed to act right through the film. The plate must then be thoroughly washed for, say, five or ten minutes, under running water, and placed in a bath of dilute ammonia (1 in 20). Finally the plate must be washed, and can then be placed on the rack to dry.

CHAPTER VII.

ON PRINTING.

The negative having been obtained and its defects, if any, remedied, the next process is its printing, and for this purpose, to our mind, none is more beautiful than carbon, which has this advantage, that it is absolutely permanent, and that the disadvantage which is usually urged, does not apply to photo-micrography—namely the reversed position of the image. We shall describe this process first, because by means of it we can both make prints and lantern slides, the latter being in the majority of cases the means taken for displaying the specimens for public demonstration; an additional advantage being that by means of this process the final image can be obtained of any colour thus rendering the specimen a nearer approximation in many cases to the original specimen. The first step is to put what is termed a safe-edge round the negative, this being most conveniently done by pasting four strips of non-actinic paper on the back of the negative so as, as it were, to make a complete frame round the image. The reason of this frame being necessary being that without it the edges of the carbon tissue would wash up during the subsequent development.

The negative is now placed in the printing frame and a piece of sensitised carbon tissue* laid upon it. The exposure of this tissue must be judged by means of an actinometer as the image is practically invisible. Several forms of actinometers are made for this purpose, consisting of a graduated scale of tints superposed on a strip of sensitised albumen paper. A trial print is made in silver and the actinometer is exposed synchronously, and when the silver print is correctly printed the number of the actinometer square, which has *just become visible*, is noted, this forms a guide for future exposures, it being only necessary to watch the actinometer. As the tissue gets older it requires slightly longer exposure; it remains in good condition for about ten days after sensitising. The print having been removed from the frame, the next step is development, which, in the case of photo-micrographs can be done on the final support at once. If the specimens are to be shown at public meetings, a very good way is to develop them upon sheets of matt surface opal glass, thus giving very brilliant results; but if the specimens are for preservation in books, they can be developed upon *autotype single transfer paper*, or if for lantern slides, upon glasses $3\frac{1}{4}$ inches square (which, after being first thoroughly cleaned), are coated with a

* The Autotype Company send out Carbon tissue freshly sensitised two or three times a week, thus saving a good deal of trouble to amateurs in sensitising the tissue themselves.

ON PRINTING.

substratum of gelatine by immersing them in a bath of

Gelatine	1 ounce
Water	10 ounces.

and subsequent drying and immersion in

Pot. bichromate	2 drachms
Water	10 ounces.

And exposure to day-light.

Whatever may be the substance of the final support, the treatment of the tissue is the same. It is immersed in a dish of cold water, and is left there until the tissue, which at first curls, begins to uncurl, the surface is then brought into contact with the support under water, and is firmly squeegeed to force into accurate contact and expel all air-bubbles; the support and tissue, now in contact, are placed to partially dry between sheets of blotting paper under pressure. After about 15 minutes they are removed and developed by immersion in warm water (*Fah.* 100°) until the pigmented gelatine is seen to ooze from the sides of the paper, which is now raised by the corner and pulled away, and the water kept moving over the surface of the print, either by shaking the dish from side to side, or by laving warm water on to the surface with the hand, the bathing in warm water being continued until the high-lights are quite clean and clear. To secure this, the water is changed two or three times, and the last bath had better be slightly warmer than its

predecessors (say *Fah.* 120°). The print is now immersed in alum solution 5%, and left there to harden for about 20 minutes, when it is rinsed in water and dried. The process takes longer to describe than to do, and it is quite easy to manipulate. Other processes, suitable for printing photo-micrographs, are

This latter process has the advantage over others that it can be used at night, the exposure being made to gas or lamp light; full instructions for working the processs are enclosed in each packet of paper. It is better for photo-micrographs to have a glazed surface as this tends to show the detail better than when the matt surface is retained. Both gelatine, bromide and chloride papers can have a high glaze placed on them by first hardening the gelatine by immersion in alum 5% solution, then squeegeeing into contact with sheets of care-cleaned *polished* plate glass—or specially enamelled iron plates sold for this purpose (these have the advantage of not needing such careful cleaning as the glasses, although the surface thus given is not so highly polished). When dry the prints strip off of their own accord.

If the prints are to be mounted on cards, and the polished surface obtained as above described is

desired to be retained, they should have a thin line of stiff glue applied round their edges to a depth of about $\frac{1}{16}$ of an inch (this being applied by means of a brush and straight-edge) and the print pressed into contact with a *warm* flat-iron. If starch is used as a mountant the water from the starch would soak into the gelatine and so destroy the glass before the print could be got on to the mount. If starch must be used it may either be applied to the print whilst it is still damp on the plate glass or enamelled iron, and then allowed to dry, and the card *only* damped for mounting purposes, or an alternative process is when the prints are all on the enamelling plate to paste them all over together whilst still damp, and place a sheet of fine thin paper over the whole of them and then peeling them all off at once, when dry. They can then be trimmed and mounted in the usual way, the extra thickness of paper preventing the gelatine surface receiving any moisture through the back whilst they are being affixed to their mounts.

As lantern slides must often of necessity be made at night, it will be as well to give a rough sketch of gelatine-bromide lantern plate manipulation. The size of a photo-micrograph renders it possible, in the majority of cases, to make lantern slides from them by contact. With average negatives and average lantern plates, an exposure of 15 to 20 seconds to gaslight of a fish-tail burner, at a distance of about one foot from the flame, will

be found ample. Development is best carried out by means of some form of Quinol developer. The following can be recommended:—

A.	Quinol	80 grs.
	Sodium sulphite	3 oz.
	Pot. bromide	20 grs.
	Metabisulphite potassium	1 drachm.
	Water	10 oz.
B.	Potassium hydrate	60 grs.
	Water	10 oz.

Mix the above solutions, A and B, in equal parts, do not wet the plate before development, as this often causes the formation of pinholes in the film. The above developer will be found to give sufficient density, and will retain the high lights absolutely pure. Development should not be carried quite so far as is desired in the finished result, as during the pouring off of the developer a wonderful increase in density will be noticed to take place. The plate is now fixed in a solution of

Sodium thiosulphate (hypo)	4 oz.
Water	20 oz.

and after thoroughly cleared it is washed as if a negative. Should the plate be too dense it can be reduced by immersion in

10 % Solution ferrocyanide of pot.:

(yellow prussiate)	30 minimis.
-----------------------------	-------------

Sodium thiosulphate solution (as above)	1 dram.
--	---------

Water	1 oz.
-------------------	-------

care being taken that too much reduction does not take place.

The slide having been allowed to dry, two little discs of paper (gummed stamp edging) are affixed, one in each upper corner on the gelatine surface of the plate, and the surface is then covered with a piece of clean glass the same size as the plate, and the two are now bound together with strips of gummed paper. The object of the discs of paper is to show which way the specimen is to be inserted in the lantern during exhibition, the discs being placed downwards and towards the condenser.

Before leaving the subject of printing we may mention that for purposes of book illustration the process, "*par excellence*," is photogravure or heliogravure, which is a half-tone intaglio copper-plate process; instructions for working this process can be found in many excellent text-books on the subject. The drawback to the process is its expense, each print costing an appreciable amount, having to be printed separately. For printing with text any good half-tone photo-zinc process may be chosen. The cost of the latter varies from 6d. to 2/- per square inch. The full-page photographs illustrating this book will serve as examples of the process.

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